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THE EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE AND ITS ZOONOTIC ASPECT IN EGYPT

By

Nahed, H. Ghoneim*; Abdel-Karim, M. Abdel-Karim*; Abdel-Moein K.A*.

Laila El-shehawyand EI sabagh,l***

* Faculty of Veterinary Medicine Cairo University, Cairo, Egypt.

** Veterinary Serum and Vaccine Research Institute, Cairo, Egypt.

ABSTRACT

Purpose: this study is carried out to investigate the epidemiology of FMD in Egypt and its zoonotic aspect.

Methods: esophageal /pharyngeal, milk and fecal samples were collected from cattle, buffaloes, sheep, goats**;** also, samples from animal drinking water and soil from animal yards were collected for both the detection of FMDV by RT-PCR and isolation of the virus on BHK including typing of isolates by ELISA. In addition, tissues of rodents trapped from animal yards were examined by both techniques. Furthermore, animal sera were tested for non structure protein (NSP) antibodies by ELISA while human sera obtained from animal contacts were tested for FMDV antibodies.

Results: FMDV was detected and isolated at the following rates (30.3 %, 18.4%, 32.6% and 45.2%) for cattle, buffaloes**,** sheep and goats respectively. The majority of isolates were from asymptomatic animals however few cases were recorded. FMDV type O was the predominant. The virus was detected in the feces of ten animals as well as in the milk of three animals while being asymptomatic. The rates for (NSP) antibodies were (28.8%, 23.4 % , 42.9 % and 30.8%) for cattle, buffaloes, sheep, goats respectively while neither rodent nor human samples was positive. Moreover, the virus was detected in five water samples but none of the soil samples were positive. Conclusion: high incidence of FMD among asymptomatic animals with shedding of the virus in saliva, feces, milk denoting the possible role of asymptomatic animals specially sheep and goats in the epidemiology of FMD in Egypt through dissemination of the virus and initiation of new epidemics putting in our mind the possibility of water borne transmissionof the virus.

On the other hand, rodent and man may have no role in the epidemiology of FMD in Egypt and so, FMD may be considered as an occasional zoonosis.

Key words: Foot and Mouth Disease, animal, epidemiology, zoonoses, Egypt.

INTRODUCTION

Foot and mouth disease was recognized many years ago, the first description of the disease was probably done in Italy in 1514 by Fracastorius. Currently, FMD distributed in many countries with outbreaks causing massive economic losses. FMD virus is a member of genus *Aphthovirus*, family *Picornaviridiae* and has seven immunologically distinct serotypes each one contains multiple subtypes. The disease affects all cloven hoofed animals including cattle, sheep, goats and pigs as well as 70 species of wild animals including deer. **(Fenner et al., 1993).** The first detection of FMD in Egypt was in 1950 when an outbreak of FMD serotype SAT2 took place. Then several outbreaks caused by serotypes O, A, SAT2 occurred during 1950s. After that serotype O became the only serotype which causes outbreaks from time to time in Egypt **(Aidaros, 2002).** In 2006, large outbreaks were reported in Egypt caused by serotype A **(Knowles et al., 2007)** while recent outbreaks in 2007, 2008 were caused by serotype O **(FAO, 2008).** The present study was done to investigate the epidemiology of FMD in Egypt including its zoonotic potential.

MATERIALS AND METHODS

Materials: - FMDV type O1 and A/ Egypt / 2006 were locally isolated from diseased animals in Egypt and were confirmed at World Reference Laboratory for FMD Pribright, U.K. (WRL). It was stored at -70 °C.

Tissue culture cell line: Baby hamster kidney cells (BHK 21 clone 13) were supplied by the animal virus institute, Pribright, U.K.

Samples:- serum , esophageal /pharyngeal (O/P), fecal and milk samples were collected from 271 animals(145 cattle, 49 buffalo (*Bubalus bubalis)* , 46 sheep and 31 goats) , in addition to tissues (liver, spleen, intestine) and feces from 19 rats trapped from the locality of the animals. Moreover, serum samples were obtained from 50 persons in intimate contact with the examined animals. Also, 52 water samples and 31 soil samples were collected from drinking containers and

yards of the animals. All animal and human samples were collected from (Kalubia, Sharkia, Giza and Fayium) governorates of Egypt.

Materials used in Enzyme linked Immunosorbant Assay (ELISA): ELISA reagents were prepared according to **(Voller et al., 1976).**

Chekit FMD-3ABC ELISA test kit (Bommeli Diagnostics, Liebefeld-Bern, Switzerland) it was used for detection of FMD non structural protein (FMD-NSP) antibodies in animal sera.

Materials for RT- PCR:

Universal Primers (POR/POF) sequences derived from 3D polymerase were designed according to **(Shin et al., 2003)** and synthesized by (Metabion. Germany).

primer	orientation	Sequence (5 to 3)	Genetic location
POF	**Forward**	**CCT ATG AGA ACA AGC GCA TC**	**3D**
POR	**Reverse**	**CAA CTT CTC CTG TAT GGT CC**	**3D**

SV total RNA isolation system for extraction of viral RNA (Promega, USA.).

QIAgen RT-PCR kit for one step RT-PCR (QIAgen, Germany).

Nucleic acid marker: - Direct load Tm wide range DNA marker 50 bp – 10000 bp was obtained from Sigma, USA.

METHODS

FMD virus isolation: - It was carried out from (O/P, milk, feces, water, soil and rats' tissue) samples collected from the examined animals by using tissue culture technique according to the directions of OIE **(OIE, 2006).** The isolates were identified by using sandwich ELISA against serotype O and A according to **(Voller et al., 1976).**

Detection of FMDV by RT-PCR:-

1-FMDV RNA extraction: It was conducted using SV total RNA isolation kit (Promega, USA) following the manufacture instructions. RNA was extracted from (O/P, milk, feces, water, soil and rats' tissues) samples.

2- One step reverse transcription polymerase chain reaction (RT-PCR):-

It was carried out according to the manufacture's protocol and OIE manual **(OIE, 2006),** the thermal profile : 50°C for 30 min; 94°C for 15 ; 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min followed by final extension at 72°C for 10 min, then electrophoresis step was

done and a positive result was noted for a sample whenever the expected size for specific bands were detected at 422 bp (Figure 1).

Chekit FMD-3ABC ELISA for detection of non structural protein antibodies in animal sera. The technique was done according to the directions of the kit. Whereas, human sera were examined by serum neutralization test for detection of FMD antibodies according to **(Ferreira, 1976).**

RESULTS

The prevalence of FMDV among examined animals was (30.3 %, 18.4%, 32.6% and 45.2 %) for cattle, buffalo, sheep and goats respectively (Table 1). Furthermore, the prevalence of serotype O was 25.1% and serotype A was 5.2% (Table2). The sero prevalence of FMDV NSP (Non Structural Protein) antibodies in examined animals was 28.8%, 23.4 %, 42.9 % and 30.8% for cattle, buffaloes, sheep and goats respectively, while all examined human sera were negative (Table 3). FMDV was detected in three milk samples out of 60 examined giving a percentage of 5% whereas the virus was detected in the feces of ten animals out of 232 examined at a ratio of (4.3%) however, none of rats' tissues yielded FMDV. About environmental samples, five out 52 water samples were positive at a ratio 9.6% but none of soil samples was positive.

DISCUSSION

The results of this study revealed high prevalence of FMD in Egypt in different animals (30.3 %, 18.4%, 32.6% and 45.2 %) for cattle, buffalo, sheep and goats respectively in examined governorates with high sero prevalence of NSP antibodies. Most of isolates obtained from asymptomatic infected animals, the majority of these animals were previously vaccinated and FMDV was detected mostly in O.P samples while milk and fecal samples were approximately negative and so these animals may be considered as carriers mentioning that carrier state in animals may last for months **(CFSPH, 2007)**, as that these vaccinated asymptomatic infected animals assumed to have a minor role in the transmission of FMD **(Orsel and Bouma 2009).** On the other hand, we detected FMDV in O.P, milk and/or fecal samples from unvaccinated sub clinically infected animals indicating that these animals which escape from vaccination may play an important role in spreading of FMD as we recorded 3 cases of FMD in cattle in the surrounding zone thus unvaccinated sub clinically infected animals may play an important role in

the initiation of new outbreaks **(Sutmoller and Casas 2002),** it is worthy to mention that most of these animals were sheep and goats. In sheep and goats the disease is usually mild and passed without detection **(Donaldson and Sellers, 2000; Ranabijuli et al. 2010),** the high prevalence of FMDV in sheep and goats obtained in the present study 32.6% and 45.2% respectively highlights the potential role which may be played by these animals in the epidemiology of FMD in Egypt especially sheep as it is highly susceptible to air borne infection (aerosols) and also can release the virus (**Kitching and Hughes, 2002).** In this study we isolated the virus from O.P, milk and feces from apparently healthy sheep indicating sub clinical infection rather than carrier state. Furthermore, sheep as grazing animals with continuous movement everywhere are able to disseminate the virus in the environment and thereby introduce it into new places; in addition we could not ignore the role of sheep in the large outbreaks of FMD in UK 2001**(Gibbens et al., 2001).** Unfortunately, sheep and goats in Egypt are usually away from vaccination and so remain susceptible for infection. Most of FMDV isolates in the study were typed as serotype O whereas serotype A was present to a lesser extends and no other FMDV serotypes were recorded. The decrement of the prevalence of serotype A in spite of large outbreaks in 2006 may be due to using of bivalent vaccine containing O1 and A/Egypt/2006 local isolates in the routine vaccination of animals however most of goats' isolates were serotype A leading us to conclude that goats may be a reservoir for FMDV serotype A in Egypt. In this study we investigated the role of rodent in the epidemiology of FMD but we found that all samples and tissues collected from rats trapped from the vicinity of the examined animals were negatives denoting that rodent may have no role in the epidemiology of FMD. Regarding to environmental samples, the detection of FMDV in animal feces which contaminate the environment as well as the isolation of the virus from animal drinking water magnifies the role of environment in the spreading of FMD considering the latest outbreaks in **2007 and 2008** in Egypt which occurred around watering and grazing points **(FAO, 2008).** The zoonotic aspect of FMD was assumed for many years and the virus was isolated and typed from diseased human cases **(Bauer, 1997).** In Egypt, **Donia and Youssef (2002)** found high sero prevalence of FMD antibodies with and without fever among persons in contact with diseased cattle during outbreak of FMD in Alexandria Governorate in Egypt 2000 indicating a zoonotic potential of FMD. Nevertheless, in the current study sera examined from all persons in intimate contact with the examined animals including diseased animals were negative when examined by SNT. In conclusion, FMD in Egypt takes

temporal pattern with appearance of outbreaks from time to time which may be attributed to presence of nuclei of sub clinically infected animals specially sheep and goats as they are usually away from vaccination and ruled out, whereas rodent and human may not play a role in the epidemiology of FMD and thus FMD may be considered as an occasional zoonosis.

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Table (1):- Prevalence of FMDV among examined animals.

Animal species	Number of examined animals	Number of positives	Percentage %
Cattle	**145**	**44**	**30.3**
Buffalo	**49**	**9**	**18.4**
Sheep	**46**	**15**	**32.6**
Goats	**31**	**14**	**45.2**
Total	**271**	**82**	**30.3**

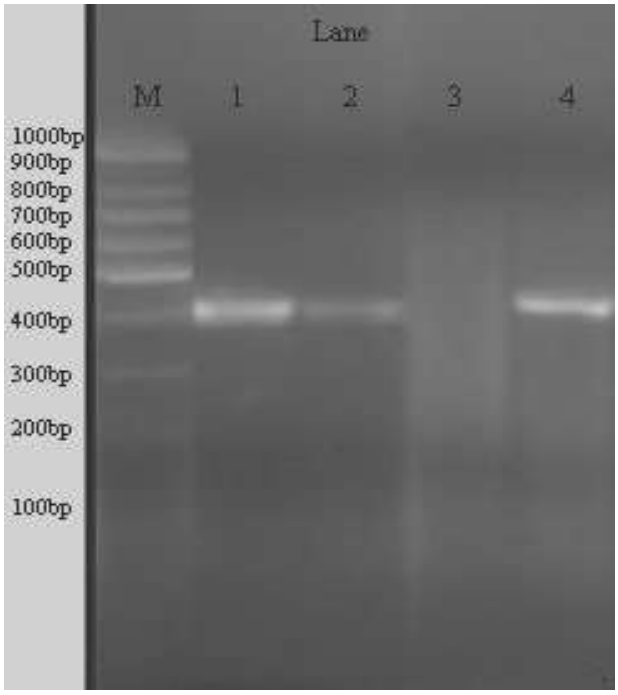
Table (2):- Prevalence of FMDV among animals examined by isolation of the virus and typed by ELISA.

Animal species	Number of examined	Positives as type O		Positives as type A	
		No.	%	No.	%
Cattle	**145**	**41**	**28.3**	**3**	**2**
Buffalo	**49**	**8**	**16.3**	**1**	**2**
Sheep	**46**	**14**	**30.4**	**1**	**2.2**
Goats	**31**	**5**	**16.1**	**9**	**29**
Total	**271**	**68**	**25.1**	**14**	**5.2**

Table (3):- seroprevalence of FMD in man and animals.

Percentage %	Number of positives	Number of examined	Animals species
Cattle	**28.8**	**40**	**139**
Buffalo	**23.4**	**11**	**47**
Sheep	**42.9**	**18**	**28**
Goats	**30.8**	**8**	**26**
Human	**0**	**0**	**50**

Figure (1): Results of examined samples by RT- PCR.



M: marker; lane 1 and 2 (+) samples of FMDV; Lane 3: (-) control; lane 4: (+) FMDV (422 bp).